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Determining the Origin of Changing β-catenin Concentrations in Zebrafish Oocytes During Maturation

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Introduction

During maturation, oocytes (immature eggs) progress from prophase I to metaphase II of meiosis, and a multitude of other cellular changes occur[1]. β-catenin is a unique protein as it is involved in cell-cell adhesion when it is bound in plasma membrane complexes, and acts as a transcription factor when freed into the cytoplasm and allowed to move into the nucleus[2]. β-catenin has been found to take part in signaling pathways such as the Wnt/β-catenin pathway, which inhibits β-catenin degradation, and subsequently regulates gene transcription[3]. β-catenin has also been found to exist in distinct molecular forms depending on its role, which may limit competition between the roles of adhesion and signaling. There is also evidence of "inactive" β-catenin, which participates in neither signaling nor adhesion[4]. Preliminary research suggests that β-catenin increases in relative cytoplasmic concentration during oocyte maturation[5]. I aimed to confirm these results by testing the hypothesis that the concentration of β-catenin in the cytoplasm increases in zebrafish oocytes during maturation. By destabilizing the cytoskeleton and freeing β-catenin into the cytoplasm with lactrunculin A, I determined if the overall concentration of β-catenin changes during maturation. Concentration changes were determined through Western blotting, and qualitative analysis was preformed through visualization with confocal microscopy. If β-catenin increases in both cytoplasmic concentration and overall amount after maturation, this would suggest that the increase is due to processes in the cytoplasm, such as a Wnt-like signaling pathway preventing the degradation of β-catenin or the new translation of protein, as opposed to the recruitment of β-catenin from the cell membrane.

Materials and Methods

- Induce maturation in oocytes with progesterone (DHP)
- Use Lactrunculin A to destabilize actin cytoskeleton
- Western blotting
  - Determine relative concentrations of β-catenin
  - Compare immature and mature oocytes and oocytes +/- Lactrunculin A
  - Use ImageJ to compare band densities and determine relative amount of protein in each sample
- Confocal microscopy
  - Stain oocytes with fluorescent anti-β-catenin
  - Visualize localization of β-catenin
  - Verify effects of Lactrunculin A

Acknowledgements

I would like to thank the University of Puget Sound, and the McCormick fund for funding my research, Krystle Pagarigan and Dan Fong for teaching me how to use the confocal microscope, and everyone else who helped me and worked with me this summer.

Results

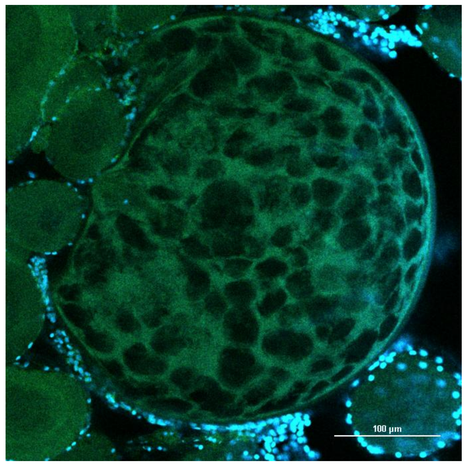

Figure 1. Zebrafish Oocyte +DHP, stained with FITC Anti- β-catenin and DAPI (Average cytoskeletal width = 5.89 um)

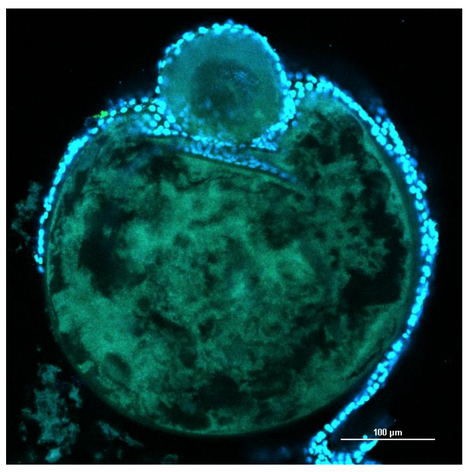

Figure 2. Zebrafish Oocyte -DHP, stained with FITC Anti- β-catenin and DAPI (Average cytoskeletal width = 5.64 um)

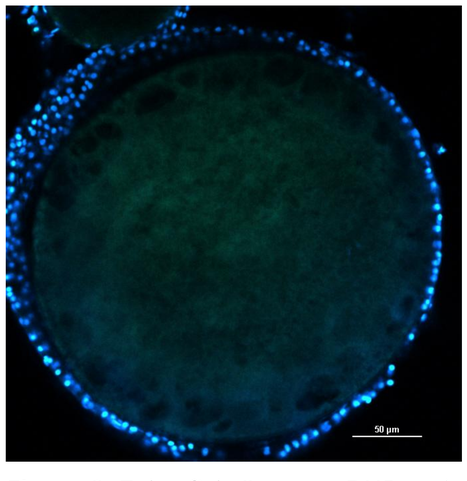

Figure 3. Zebrafish Oocyte +DHP and +Latrunculin A, stained with FITC Anti-β-catenin and DAPI (Average cytoskeletal width = 4.66 um)

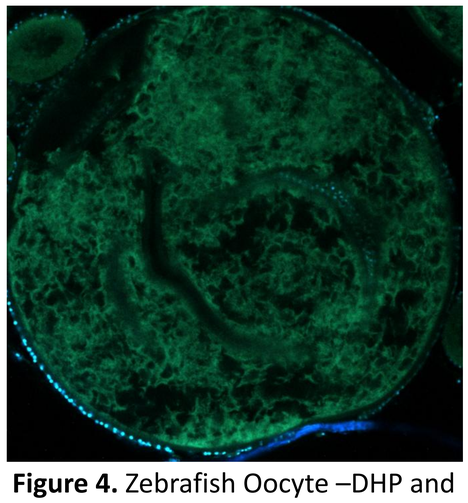

Figure 4. Zebrafish Oocyte –DHP and +Latrunculin A, stained with FITC Anti-β-catenin and DAPI (Average cytoskeletal width = 4.73 um)

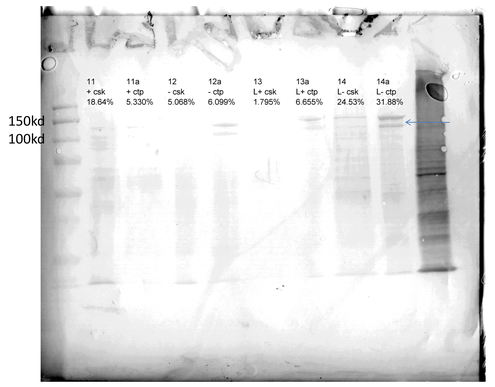

Figure 5. Western Blot of Cytoskeletal and Cytoplasmic Proteins with Densomitry Analysis. Blot was prepared using anti-β-catenin antibody.

Discussion

- Results of Western blot analysis proved inconclusive. The only successful blot was too inconsistent to be considered reliable.
- Confocal analysis showed first that the staining procedures for both FITC anti-β-catenin and DAPI were successful. In addition, β-catenin appears to be distributed throughout the entire cytoplasm, except where it is possibly obscured by yolk proteins.
- Unfortunately, confocal analysis can not determine or compare total or cytoplasmic β-catenin content. However, by measuring the band of signal around the edge of the cells, cytoskeletal protein can be approximated. This showed that no significant change in cytoskeletal β-catenin occurred during maturation, suggesting that any change in cytoplasmic concentration would not be due to migration.
- The cytoskeletal width of the +Lactrunculin A samples were significantly smaller than their –Lactrunculin A counterparts, suggesting that Lactrunculin A successfully dissociated β-catenin from the cytoskeleton (t-test; p=0.012).

Future Directions

- Examine the effects of increasing or inhibiting cytoplasmic β-catenin on the processes of oocyte maturation
- Look for the presence of new β-catenin translation during maturation, possibly by examining the amount of β-catenin mRNA present before, during, and after maturation
- Look for the presence of Wnt signaling or parts of known Wnt pathways during maturation

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